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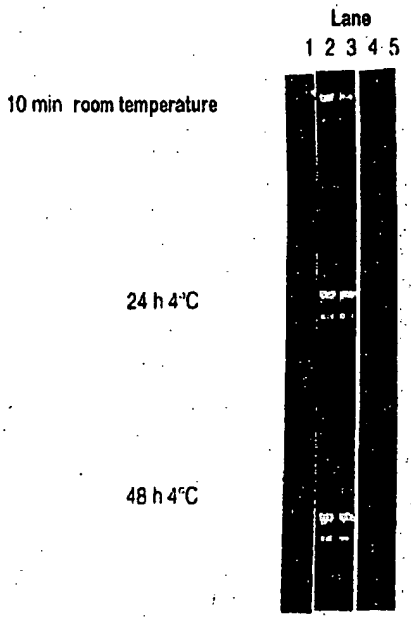
(54) UNE METHODE POUR STABILISER ET/OU ISOLER DES ACIDES NUCLEIQUES
(54) A METHOD OF STABILIZING AND/OR ISOLATING NUCLEIC ACIDS

(57)

The present invention relates to a method of stabilizing and/or isolating nucleic acids, wherein a biological sample containing nucleic acids is contacted with a cationic compound. The invention also relates to said cationic compound per se and to the use of said cationic compound in stabilizing and/or isolating nucleic acids. Furthermore, the invention relates to pharmaceutical compositions, diagnostic compositions, and to compositions used in research, which include cationic compounds or a complex being formed upon contact of said cationic compound with a nucleic acid.



Abstract:

The present invention relates to a method of stabilizing and/or isolating nucleic acids, wherein a biological sample containing nucleic acids is contacted with a cationic compound. The invention also relates to said cationic compound *per se* and to the use of said cationic compound in stabilizing and/or isolating nucleic acids. Furthermore, the invention relates to pharmaceutical compositions, diagnostic compositions, and to compositions used in research, which include cationic compounds or a complex being formed upon contact of said cationic compound with a nucleic acid.

methods such as real-time reverse transcriptase PCR (real-time RT PCR) or gene expression chip analyses permits e.g. the recognition of incorrectly expressed genes, thereby allowing the recognition of e.g. metabolic diseases, infections, or the development of cancer. By analyzing the DNA from cells using molecular-biological methods such as PCR, RFLP, AFLP or sequencing, it is possible e.g. to detect genetic defects or to determine the type of HLA and other genetic markers.

The analysis of genomic DNA and RNA is also used in the direct detection of infectious pathogens such as viruses, bacteria, etc..

One indispensable precondition for nucleic acid analytics is immediate stabilization of the nucleic acids after the biological sample has been extracted from its natural environment. This applies for DNA and RNA, particularly for RNA which may undergo exceedingly rapid degradation once the biological sample has been extracted. On the other hand, extraction of the biological sample may be followed by synthesis of new mRNA molecules as a result of e.g. induction of stress genes, so that the transcript pattern of the cells could be changed. In this way, subsequent analyses may be distorted.

To date, it is barely possible to stabilize nucleic acids, particularly over a prolonged period of time, e.g. for several hours or days and up to weeks when using means suitable for routine analyses. This is highly disadvantageous because, e.g. in the medical field, e.g. in a medical practice, it is often the case that samples containing nucleic acids are collected which can be put to further examination only after prolonged storage and transportation to a laboratory.

In the meantime, the nucleic acids contained in the

There is a number of methods of isolating nucleic acids where the cell is destroyed and RNA and/or DNA is liberated into solution. As a rule, well-known procedures of isolating nucleic acids from complex materials such as blood, serum, urine, or feces comprise lysis of the biological material by means of a detergent in the presence of proteinases, followed by multiple extractions using organic solvents such as phenol and/or chloroform, ethanol precipitation, and dialysis of the nucleic acids. Procedures of this type have been described by e.g. Chirgwin et al., Biochem. 18, 5294-5299 (1979), D.M. Wallace in Meth. Enzym. 152, 33-41 (1987), P. Chomczynski and N. Sacchi, Anal. Biochem. 162, 156-159 (1987), and "Preparation and Analysis of RNA" in Current Protocols in Molecular Biology, Unit 4.2 (Supplement 14), editor: F.M. Ausubel et al., John Wiley (1991), T. Maniatis et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbour Laboratory (1992); L.G. Davis et al., "Guanidine Isothiocyanate Preparation of Total RNA" and "RNA Preparation: Mini Method" in Basic Methods in Molecular Biology, Elsevier, N.Y., pages 130-138 (1991), and in US patent No. 4,843,155 to Chomczynski.

Furthermore, it is familiar to isolate nucleic acids from various starting materials by mixing the starting material with a chaotropic substance and a solid phase which binds the nucleic acid. In a subsequent step, the solid phase is separated from the liquid and washed. If necessary, the nucleic acids can be eluted from the solid phase (US 5,234,809).

Frequently, these well-known methods of isolating nucleic acids from biological materials are exceedingly laborious and time-consuming. The number of steps - mostly being relatively large - required to purify the nucleic acids from such starting materials increases the risk of transferring nucleic acids from sample to sample when processing miscellaneous clinical samples at the same time.

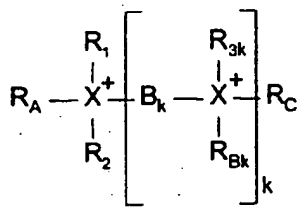
scription is inhibited by high concentrations of carrier. Also, MacFarlane as well describes a lower sensitivity in the detection of HCV in plasma (with no carrier) as compared to blood (Schmidt et al. J. Med. Virol. 47, 153-160 (1995)). In the absence of high amounts of nucleic acid, the sensitivity is very poor. In US 5,300,635, MacFarlane also describes the sedimentation of RNA-detergent complexes by centrifuging at high g values (16,000 x g in Examples 4, 5 and 6). Also, it has been demonstrated that centrifugation at low g values is not sufficient to sediment RNA-tetradecyltrimethylammonium oxalate complexes from plasma. In order to purify viral RNA from large volumes of plasma or serum (>1 ml), it is absolutely necessary to achieve sedimentation of the nucleic acid-detergent complexes at low g values because otherwise, costly and complex ultracentrifuges must be used instead of simple laboratory centrifuges (having a maximum achievable g values of 5,000-6,000).

In the embodiments in US 5,300,635, MacFarlane describes the addition of at least 2 volumes and up to 10 volumes of detergent to the sample. Thus, the total volume to be processed is considerably increased in some cases, particularly when reflecting the purification of nucleic acids from several milliliters of sample material (e.g. plasma pools). However, processing large volumes is unfavorable, particularly with respect to an optional automatization of sample preparation on a pipetting robot because the pipetted volumes are limited, for example.

Therefore, a method of stabilizing and/or isolating nucleic acids is required that would not involve the above-mentioned drawbacks of prior art.

More specifically, a method is required which permits stabilization of nucleic acids and/or lysis of a sample containing the nucleic acids and isolation of the

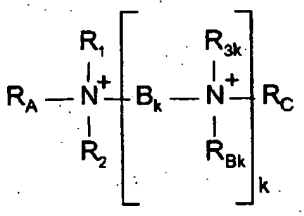
in order to stabilize and/or isolate nucleic acids:



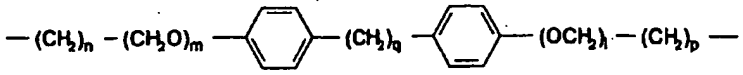
(I)

Within the scope of the present invention, the term "cationic compound" is understood to indicate a compound having more than one positive charge. The cationic compound depicted as formula (I) is used in a dissolved form and/or in the form of a salt, with charge neutralization being effected by the conjugated bases of strong and/or weak inorganic and/or organic acids, which will be abbreviated as "A" hereinafter. Consequently, the product of charge and number of bases will exactly compensate the positive charges of the rest of the compound.

In the above formula (I), X represents nitrogen atoms (N) or phosphor atoms (P). In formula (Ia), the cationic compound is shown were X = N, and in formula (Ib) the cationic compound is shown were X = P.

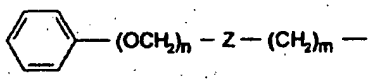


(Ia)



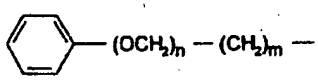
wherein n, m, l, p, q are independent of each other and represent one of the integers 0, 1, 2, 3, 4, 5, or 6. In addition, the phenyl, naphthyl or biphenyl bridge may be substituted on one or more carbon atoms.

In addition, R_1, R_2, R_{3k} in formula (I) illustrated above, which may be identical or different and which may be unsubstituted or substituted on one or more carbon atoms, represent hydrogen, linear or branched C_1-C_6 alkyl, linear or branched C_1-C_6 alkenyl, linear or branched C_1-C_6 alkynyl, phenyl, benzyl, phenoxyethyl having the structure



wherein n, m independently represent the integers 0, 1, 2, 3, 4, 5, or 6, and Z represents one of the structures -O-, -CO-, $-CO_2-$, -OCO-, -CO-N-, -N-CO-, -O-CO-N-, -N-CO-O-, -S-, or -S-S-.

Moreover, R_1, R_2, R_{3k} may represent phenyl, benzyl, phenoxyethyl having the structure



wherein n, m independently represent the integers 0, 1, 2, 3, 4, 5, or 6.

R_A, R_{Bk}, R_C, which may be identical or different and

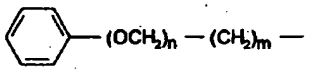
and/or isolation of the nucleic acids in one single step. The stabilized nucleic acids not only are stable during the preparation but also over a prolonged period of time, such as 96 hours or more. In particular, the complexes consisting of nucleic acid and cationic compound can be sedimented at low g values, where only low amounts of carrier nucleic acids or carrier aids are required, or even none at all, and where only small volumes or amounts of cationic compound must be added to the sample. In addition, owing to the pelletizing of the complexes, it is possible to work in small volumes as early as after this step.

As a result of the inventive stabilization of nucleic acids, the nucleic acids in a sample do not change their structure even upon prolonged storage or during transportation, and the accuracy of tests to be conducted at a later time is significantly increased. In some cases, e.g. where materials containing nucleic acids have to be shipped over long distances or subjected to prolonged storage, such tests are actually made possible by the method of the invention.

The compound may be added in solution or as a solid. The option of adding as a solid involves the additional advantages that solids mostly have higher chemical stability and their addition to the sample frequently can be done more easily. It is possible to add one cationic compound or a mixture of two or more cationic compounds.

The method according to the invention preferably uses compounds of general formula (I) specified above, where an anion A selected from the group of fluoride, chloride, bromide, iodide, perchlorate, perbromate, periodate, phosphate, hydrogen phosphate, dihydrogen phosphate, sulfate, thiosulfate, hydroxide, carboxylic acids, α-halocarboxylic acids, and/or hydroxycarboxylic acids is used, and k represents the integer 1, 2, 3, 4, 5, or 6,

dimethyl-2-butynyl, 1,1-dimethyl-2-butynyl, 1,1-dimethyl-3-butynyl, 1,2-dimethyl-3-butynyl, 1,3-dimethyl-2-butynyl, 2,2-dimethyl-3-butynyl, 1-ethyl-2-butynyl, 1-ethyl-3-butynyl, 2-ethyl-3-butynyl, and/or 1-ethyl-1-methyl-2-propynyl, and/or the groups benzyl, phenylethyl, phenylpropyl, phenylisopropyl, phenylisobutyl, phenoxymethyl, phenoxyethyl, phenoxypropyl, phenoxyisopropyl, phenoxybutyl, phenoxyisobutyl having the structure



wherein n, m independently represent the integers 0, 1 or 2.

The residues R_A, R_{Bk}, R_C, which may be identical or different, represent the linear or branched C_8-C_{20} alkyl groups octyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, and/or eicosyl, and/or the linear or branched C_8-C_{20} alkenyl groups octenyl, decenyl, undecenyl, dodecenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl, octadecenyl, nonadecenyl, and/or eicosenyl, and/or the linear or branched C_8-C_{20} alkynyl groups octynyl, decynyl, undecynyl, dodecynyl, tridecynyl, tetradecynyl, pentadecynyl, hexadecynyl, heptadecynyl, octadecynyl, nonadecynyl, and/or eicosynyl, and/or a structure

$$CH_3-(CH_2)_n-Z-(CH_2)_m-$$

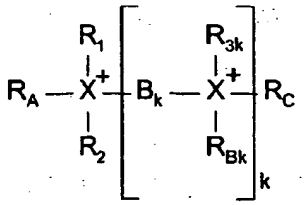
wherein n, m are independent of each other, and n represents the integer 2, 3 or 4, m represents the integer 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18, and Z represents one of the structures -O-, -CO-, -OCO-, -CO-N-, or -N-CO-.

Within the scope of the present invention, it is preferred to use compounds of general formula (I) wherein one or more of the groups designated as R_A, R_{Bk} and R_C

1,2-diyl, propane-1,1-diyl, propane-1,2-diyl, propane-1,3-diyl, butane-1,1-diyl, butane-1,2-diyl, butane-1,3-diyl, and/or butane-1,4-diyl. R_1, R_2, R_{3k} represent methyl, ethyl or hydroxyethyl, while R_A, R_{Bk}, R_C represents the linear C_8-C_{20} alkyl groups octyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, and/or eicosyl.

In the method according to the invention, it is particularly preferred to use compounds of general formula (I) wherein the residues R_1, R_2 and R_{3k} are identical and/or R_A, R_{Bk} and R_C are identical and/or if $k > 1$, the bridging groups B_k are identical.

In all the compounds used according to the invention, the carbon atoms in the groups R_1, R_2, R_{3k}, R_A, R_{Bk}, and R_C may be substituted with one or more halogen atoms, particularly one or more fluorine atoms, and/or one or more primary, secondary and/or tertiary hydroxyl groups, and/or one or more -SH, $-NH_2$, -NH-, and/or =N- groups, where the substituents may be identical or non-identical to each other. Those compounds are preferred wherein the distance between the first substituted carbon atom and the nitrogen drawn in general formula (I) is at least two covalent bonds. As a consequence, one or more carbon atoms of the groups R_1, R_2, R_{3k}, R_A, R_{Bk}, and R_C, which are not directly bound to one of the atoms (nitrogen or phosphor) in compound



(I)

are substituted.

After contacting the cationic compound with a biological sample, the cationic compound can be mixed with the biological sample, and the mixture can be incubated, incubation preferably being performed for 10 minutes at room temperature.

According to a preferred embodiment of the present invention, the cationic compound and/or the complex formed of nucleic acid and cationic compound may be added with additional means to support lysis. Alcohols, particularly branched and unbranched C1- to C4-alkanols like isopropanol, aldehydes particularly lower C1- to C4-aldehydes, branched or unbranched such as glyoxal, phenols, phenol derivatives such as 2-biphenylol, ionic, zwitterionic and non-ionic compounds, reagents reducing sulfhydryl, particularly dithiothreitol, phosphoric acid derivatives, particularly tributyl phosphate, chaotropic reagents such as urea, carboxylic acids, such as citric acid or malonic acid, or plain salts, such as ammonium salts or alkali phosphates, can be used alone or in combination as agents to support lysis.

According to another preferred embodiment of the present invention, it is also possible to homogenize the biological sample or subject it to mechanical or enzymatic exposure prior to or during addition of the cationic compound. For example, mechanical exposure might be effected using an electric knife, a ball mill, addition of particles, or by pressing through a syringe, while suitable enzymes to act upon the sample might be hydrolases, proteases or lipases, for example. Other options are well-known to those skilled in the art and are encompassed herein. Such treatment of a biological sample might be advantageous in that the cationic compound has a better chance of contacting its targets of attack.

According to the invention, the complexes formed of

by reference), and subjected to further purification. If the complexes are dissolved under non-binding conditions, the nucleic acids can be collected in a collecting tube by means of centrifugation, vacuum, or excess pressure. If necessary, they may then be subjected to further purification in relatively small volumes, using various well-known procedures. Thus, for example, it is obviously possible - once appropriate binding conditions have been adjusted - to rebind them on a membrane or another surface for further purification.

Sample materials free of cells, food samples containing free or bound nucleic acids or nucleic acid-containing cells, environmental samples containing free or bound nucleic acids or nucleic acid-containing cells, suspensions of cells, bacteria, viruses, or yeasts, any type of tissue or clinical samples such as blood, plasma, serum, leukocyte fractions, *Crusta phlogistica*, sputum, urine, sperm, feces, or smears, as well as plants or plant parts or free nucleic acids can be used as biological samples including nucleic acids, as well as any other imaginable sample which contains nucleic acids.

According to the invention, the above-specified cationic compounds are used in a kit for stabilizing and/or isolating nucleic acids, which kit preferably includes additional suitable buffers. In addition, the kit may include suitable means to support lysis and/or means for purifying the nucleic acids and/or means for mechanical exposure and/or means for enzymatic treatment of the samples and/or complexes.

According to the invention, the above-specified cationic compounds are used to stabilize and/or isolate nucleic acids, a complex being formed which consists of a nucleic acid and a cationic compound. Said complex is remarkable for its particularly advantageous, high stability, thereby pro-

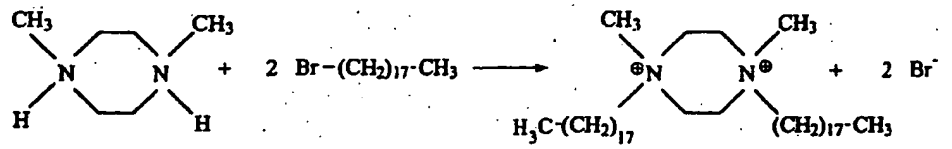
Linear, branched and cyclic cationic compounds are prepared according to Examples 1 or 2. To bind the residues R_A and R_{Bk} (if $k = 1$, R_C will be used instead of R_{Bk}) to the nitrogen atoms by nucleophilic substitution, tertiary diamines or tertiary polyamines ($k > 1$) having a predetermined number of tertiary nitrogen atoms were added with an excess of alkyl halide in solution under argon protective gas. The nitrogen atoms are linked by linear (unbranched) alkanediyl bridges or substituted xylylene bridges having the appropriate length n. This *per se* known quaternization reaction was conducted at elevated temperatures. Alkyl halides such as alkyl bromide or alkyl iodide were used in excess to prepare ammonium salts, most of which were completely quaternized. The ammonium compounds thus obtained were purified by recrystallization from various solvents and solvent mixtures such as diethyl ether/methanol.

Alternatively, cationic compounds having two cationic nitrogen atoms ($k = 1$) were synthesized. To this end, primary α,ω-alkanyl dihalides were reacted with an excess of alkyldimethylamine under the reaction conditions according to Example 1. The alkyl chain of the amine compound may be hydroxylated but has no halogen atoms. The cationic compounds are purified as described above.

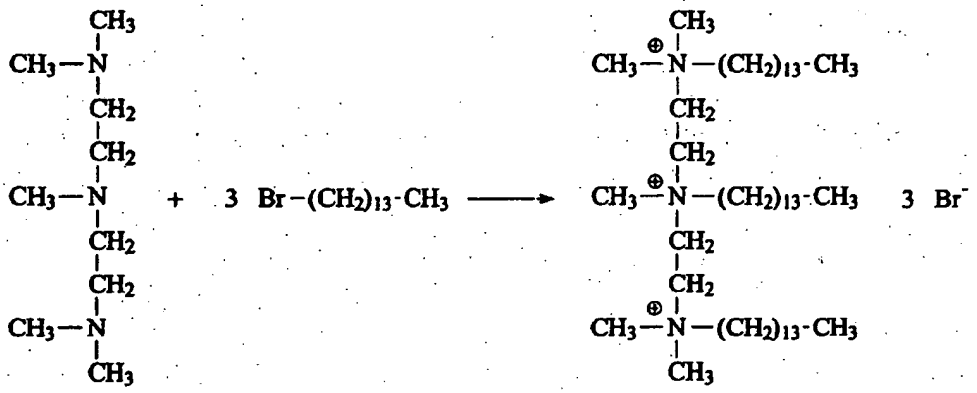
The counterions (anions A) can be exchanged using an ion exchange column. Example 3 exemplifies the exchange of bromide for acetate.

Example 1: Synthesis of ethanediyl-1,2-bis(dimethyldecylammonium bromide)

reaction equation of the preparation of N,N'-dioctadecyl-N,N'-dimethylpiperazine-diium dibromide from 1,4-dimethylpiperazine and octadecyl bromide is given as an example:



Example 2: Synthesis of N,N',N"-tritetradecyl-N,N,N',N",N"-pentamethyl-bis(2-ammonioethyl)ammonium bromide



In a 2 l round-bottom flask equipped with reflux condenser, heating jacket and magnetic stirrer, a solution of 20.9 ml of N,N,N',N',N"-pentamethyldiethylenetriamine (17.3 g, 0.10 mol) and 93.5 ml of 1-bromotetradecane (99.8 g, 0.36 mol, 20% excess) in 500 ml of acetonitrile and 150 ml of acetone was heated to reflux temperature for 72 hours.

Thereafter, the reaction mixture was cooled to room temperature and stored at 4°C overnight in order to complete

Educts		Reaction time	Yield	Product
Tetramethylethylenediamine	Octyl bromide	42 hours	45%	Ethanediyl-1,2-bis(octyldimethyl-ammonium bromide)
Tetramethylethylenediamine	Decyl bromide	42 hours	65%	Ethanediyl-1,2-bis(decyldimethyl-ammonium bromide)
Tetramethylethylenediamine	Dodecyl bromide	42 hours	63%	Ethanediyl-1,2-bis(dodecyldimethyl-ammonium bromide)
Tetramethylethylenediamine	Tetradecyl bromide	42 hours	35%	Ethanediyl-1,2-bis(tetradecyldimethyl-ammonium bromide)
Tetramethylethylenediamine	Hexadecyl bromide	42 hours	41%	Ethanediyl-1,2-bis(hexadecyldimethyl-ammonium bromide)
Tetramethylethylenediamine	Octadecyl bromide	42 hours	14%	Ethanediyl-1,2-bis(octadecyldimethyl-ammonium bromide)
1,4-Dimethylpiperazine	Octadecyl bromide	42 hours	42%	N,N'-Dioctadecyl-N,N'-dimethylpiperazine-dium dibromide
Tetramethylpropanediamine	Decyl bromide	42 hours	77%	Propanediyl-1,3-bis(decyldimethyl-ammonium bromide)
Tetramethylpropanediamine	Dodecyl bromide	42 hours	85%	Propanediyl-1,3-bis(dodecyldimethyl-ammonium bromide)
Tetramethylpropanediamine	Tetradecyl bromide	42 hours	55%	Propanediyl-1,3-bis(tetradecyldimethyl-ammonium bromide)
Tetramethylpropanediamine	Hexadecyl bromide	42 hours	91%	Propanediyl-1,3-bis(hexadecyldimethyl-ammonium bromide)
Tetramethylpropanediamine	Octadecyl bromide	42 hours	87%	Propanediyl-1,3-bis(octadecyldimethyl-ammonium bromide)
Tetramethylbutanediamine	1-Bromo-3-methylbutane	42 hours	98%	Ethanediyl-1,2-bis(3-methylbutyldimethyl-ammonium bromide)
Tetramethylbutanediamine	Decyl bromide	42 hours	78%	Butanediyl-1,4-bis(decyldimethyl-ammonium bromide)
Tetramethylbutanediamine	Dodecyl bromide	42 hours	82%	Butanediyl-1,4-bis(dodecyldimethyl-ammonium bromide)
Tetramethylbutanediamine	Tetradecyl bromide	42 hours	58%	Butanediyl-1,4-bis(tetradecyldimethyl-ammonium bromide)

				ammonium bromide)
1,2-Dibromoethane	Tetradecylamine	48 hours	53%	Ethanediyl-1,2-bis(tetradecyldimethyl-ammonium bromide)
1,2-Dibromoethane	Hexadecylamine	48 hours	50%	Ethanediyl-1,2-bis(hexadecyldimethyl-ammonium bromide)
1,2-Dibromoethane	Octadecylamine	48 hours	48%	Ethanediyl-1,2-bis(octadecyldimethyl-ammonium bromide)
1,3-Dibromopropane	Decylamine	48 hours	68%	Propanediyl-1,3-bis(decyldimethyl-ammonium bromide)
1,3-Dibromopropane	Dodecylamine	48 hours	65%	Propanediyl-1,3-bis(dodecyldimethyl-ammonium bromide)
1,3-Dibromopropane	Tetradecylamine	48 hours	63%	Propanediyl-1,3-bis(tetradecyldimethyl-ammonium bromide)
1,3-Dibromopropane	Hexadecylamine	48 hours	64%	Propanediyl-1,3-bis(hexadecyldimethyl-ammonium bromide)
1,3-Dibromopropane	Octadecylamine	48 hours	60%	Propanediyl-1,3-bis(octadecyldimethyl-ammonium bromide)
1,4-Dibromobutane	Decylamine	48 hours	65%	Butanediyl-1,4-bis(decyldimethyl-ammonium bromide)
1,4-Dibromobutane	Dodecylamine	48 hours	66%	Butanediyl-1,4-bis(dodecyldimethyl-ammonium bromide)
1,4-Dibromobutane	Tetradecylamine	48 hours	63%	Butanediyl-1,4-bis(tetradecyldimethyl-ammonium bromide)
1,4-Dibromobutane	Hexadecylamine	48 hours	65%	Butanediyl-1,4-bis(hexadecyldimethyl-ammonium bromide)
1,4-Dibromobutane	Octadecylamine	48 hours	60%	Butanediyl-1,4-bis(octadecyldimethyl-ammonium bromide)

Example 4: Reference Example

A radiolabelled *in vitro* transcript of the mouse Evx gene 4.5 kb in length was used as a model for the isolation of viral RNA from plasma. Radiolabelling was performed by incorporating α^{32}P-UTP in the RNA transcript using T7 RNA polymerase.

Amount of carrier (µg)	RNA in supernatant (%)	RNA in eluate (%)
0	83	3
2.5	79	13
5	63	26
7.5	49	39
10	30	55
25	5	75

Experiment B

2 ml of a 3.6% solution of tetradecyltrimethylammonium oxalate is added to 1 ml plasma in a 15 ml reaction vessel. Carrier RNA (poly A RNA having a length of 700 bases up to 7 kb) in varying amounts and radiolabelled transcript are placed in the cap of the reaction vessel. The cap of the reaction vessel is secured, the sample is mixed thoroughly and incubated for 10 min at room temperature. The complexes consisting of RNA and cationic compound are sedimented for 2 min at about 4,500 x g.

Thereafter, the amount of radiolabelled transcript in sediment and supernatant is determined by measurement in a scintillation counter.

Table 2: Amount of radiolabelled RNA (%) in the sediment as a function of amount of carrier and centrifugation time. The difference to make 100% results from the amount of RNA in the supernatant.

4,500 x g.

Thereafter, the amount of radiolabelled transcript in sediment and supernatant is determined by measurement in a scintillation counter.

Table 3: Amount of radiolabelled RNA (%) in the sediment as a function of amount of carrier and centrifugation time. The difference to make 100% results from the amount of RNA in the supernatant.

Amount of carrier (µg)	RNA in sediment (%)
0	95%
5	96%
10	94%

Despite low amounts of carrier or even none at all, and despite sedimentation of the complexes consisting of RNA and cationic compounds at low g values, a high yield of RNA in the sediment is obtained.

Example 6: Concentrating the complexes consisting of RNA and cationic compounds on various membranes

200 µl of plasma is mixed with 200 µl of a 1% solution of ethanediyl-1,2-bis(dimethyldecylammonium bromide). Radiolabelled transcript (see Example 5) is placed in the cap of the reaction vessel. No additional carrier RNA is added. The cap of the reaction vessel is secured, the sample is mixed thoroughly and incubated for 10 min at room temperature. The complexes consisting of RNA and cationic compound are concentrated on various membranes by passing them through these membranes using centrifugation for 2 min

with 1 ml of lysis buffer which, in addition to 1-20% (w/v) ethanediyl-1,2-bis(dimethyldecylammonium bromide), contains urea at a concentration of 1-6 M, and/or tributyl phosphate at a concentration of 0.1-1% (v/v), and/or dithiothreitol at a concentration of 5-40 mM, and/or isopropanol at a concentration of 10-50% (w/v). Radiolabelled transcript and 10 µg of poly A carrier RNA (see Example 4) are pipetted into the cap of the reaction vessel, the cap is secured, and the batch is mixed thoroughly. The batch is incubated for 10 min at room temperature. The complexes of RNA and cationic compound are sedimented in an Eppendorf 5417 centrifuge for 3 min at 3,000 rpm = about 1000 x g, and the supernatant is pipetted off. The pellet is dissolved in 500 µl of a trishydroxymethylaminomethane (Tris HCl) buffer having a pH value of 6-8 and a high salt concentration, e.g. 2-5 M LiCl, 2-5 M sodium acetate, 4-6 M guanidinium thiocyanate or 2-6 M guanidine hydrochloride (GuHCl). For improved resuspending of the pellet, the buffer may be heated to 60°C. Furthermore, proteinase K (400 µg) may be added to the buffer, and the batch may then be incubated for 10 min at 60°C. Subsequently, 500 µl of a solution is added which contains 40-98% (v/v) ethanol. In addition, one or both of these solutions may contain a non-ionic or zwitterionic detergent such as Triton X-100, Nonidet-P40, TWEEN 20, CHAPSO, or ZWITTERGENT 3-12 at a concentration ranging from 1 to 20%. The solution is loaded on a spin column containing a silica membrane and passed through the membrane using centrifugation for 1 min at about 3,700 x g. The spin column is washed twice with 700 µl of a buffer containing ethanol and NaCl, the wash buffer being passed through the membrane using centrifugation at 10,000 x g. The spin column is centrifuged to dryness for 3 min at 20,000 x g, and the RNA is eluted in two steps from the silica membrane using 30 µl of water each time.

During the procedure, all the fractions

50 mM Tris HCl pH 7.0, 3 M urea, 0.2% (v/v) tributyl phosphate	80% (v/v) ethanol, 10% (v/v) Nonidet P40	
5% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 3 M urea, 0.6% (v/v) tributyl phosphate	6 M GuHCl 50 mM Tris HCl pH 7.0 80% (v/v) ethanol, 10% (v/v) Nonidet P40	50%
5% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 3 M urea, 0.8% (v/v) tributyl phosphate	6 M GuHCl 50 mM Tris HCl pH 7.0 80% (v/v) ethanol, 10% (v/v) Nonidet P40	36%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 3 M urea, 30% (v/v) isopropanol	6 M GuHCl 50 mM Tris HCl pH 7.0 5% (v/v) Nonidet P40 80% (v/v) ethanol, 10% (v/v) Nonidet P40	66%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 3 M urea, 40% (v/v) isopropanol	6 M GuHCl 50 mM Tris HCl pH 7.0 5% (v/v) Nonidet P40 80% (v/v) ethanol, 10% (v/v) Nonidet P40	49%
2% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 3 M urea, 30% (v/v) isopropanol 10 mM Dithiothreitol	6 M GuHCl 50 mM Tris HCl pH 7.0 5% (v/v) Nonidet P40 80% (v/v) ethanol, 10% (v/v) Nonidet P40	65%
2% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 4 M urea, 30% (v/v) isopropanol 5 mM Dithiothreitol, 0.3% (v/v) tributyl phosphate	6 M GuHCl 50 mM Tris HCl pH 7.0 5% (v/v) Nonidet P40 80% (v/v) ethanol, 10% (v/v) Nonidet P40	71%
2% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.0, 4 M urea, 30% (v/v) isopropanol 5 mM Dithiothreitol, 0.3% (v/v) tributyl phosphate	6 M GuHCl 50 mM Tris HCl pH 7.0 1% (v/v) Nonidet P40, 400 µg proteinase K 80% (v/v) ethanol, 10% (v/v) Nonidet P40	78%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	2 M LiCl 50 mM Tris HCl pH 7.5 80% (v/v) ethanol	39%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	5 M LiCl 50 mM Tris HCl pH 7.5 80% (v/v) ethanol	38%
2% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	2 M sodium acetate pH 6.5 70% (v/v) ethanol	31%
2% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	4 M sodium acetate pH 6.5 70% (v/v) ethanol	30%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	4 M guanidinium thiocyanate, 50 mM Tris HCl pH 7.0 80% (v/v) ethanol	59%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	6 M guanidinium thiocyanate, 50 mM Tris HCl pH 7.0 80% (v/v) ethanol	46%
1% Ethanediyl-1,2-bis(dimethyldecylammonium bromide), 50 mM Tris HCl pH 7.5	5.5 M guanidinium thiocyanate, 40 mM sodium citrate pH 7.5, 1% (v/v) Triton X-100, 98% (v/v) ethanol	34%

guanidinium thiocyanate, 0.2 M sodium acetate and 10% (v/v) Nonidet P40. Thereafter, 100 µl of acidic phenol is added, and the solution is extracted by vigorous agitation. Following addition of 100 µl of chloroform, the solution is extracted once more by vigorous agitation and centrifuged for 1 min at 20,000 x g to effect phase separation. The aqueous phase is removed and re-extracted with 100 µl of chloroform as described above. The aqueous phase is removed, and the nucleic acids are precipitated by adding 200 µl of isopropanol over 30 min at -20°C. The precipitated nucleic acids are sedimented by centrifuging for 5 min at 20,000 x g, the supernatant is removed, and the nucleic acid sediment is washed once with an 80% ethanol solution, dried and dissolved in distilled water free of RNase.

The amount of isolated nucleic acid is determined by measuring the light absorption at a wavelength of 260 nm, and the purity of the nucleic acid is established by determining the ratio of light absorption at 260 nm and 280 nm (see Table 6).

Table 6: RNA yield and purity when using 1×10^7 HeLa cells. To determine the yield, the calculation factor for RNA is used (1 OD_{260nm} = 40 µg/ml), the OD measurement is performed in water. A triple determination is carried out.

Sample No.	Yield (µg)	OD 260nm/280nm
1	51.2	1.85
2	135	1.66
3	77.9	1.69

The result conforms to the expected amount of total RNA that could be isolated from 10^7 HeLa cells.

RNA is used (1 OD_{260nm} = 40 µg/ml), the measurement is performed in water. A triple determination is carried out.

Sample No.	Yield (µg)	OD 260nm/280nm
1	220	1.31
2	207	1.90
3	256	2.26

Example 10

Purification of RNA from plasma by complexing with cationic compounds and subsequent phenol/chloroform extraction

As a model for viral RNA (e.g. HCV or HIV RNA), HeLa RNA is added to a mixture of 140 µl of blood plasma and 140 µl of a solution of 2% (w/v) ethanediyl-1,2-bis(dimethyldecylammonium bromide), buffered with 50 mM Tris-HCl pH 7.0, and subsequently incubated for 10 min. The solution then is centrifuged for 3 min at about 1000 x g.

The supernatant is removed, and the sediment is dissolved in 200 µl of a solution consisting of 4 M guanidinium thiocyanate, 0.2 M sodium acetate and 10% (v/v) Nonidet P40. Thereafter, 100 µl of acidic phenol is added, and the solution is extracted by vigorous agitation. Following addition of 100 µl of chloroform, the solution is extracted once more by vigorous agitation and centrifuged for 1 min at 20,000 x g to effect phase separation. The aqueous phase is removed and re-extracted with 100 µl of chloroform as described above. The aqueous phase is removed, and the nucleic acids are precipitated by adding 200 µl of isopropanol over 30 min at -20°C. The precipitated nucleic acids are sedimented by centrifuging for 5 min at

hydrochloride, 50 mM Tris-HCl, pH 7.0, and 1% (v/v) Nonidet P40. Following addition of 300 µl of a solution of 80% ethanol and 10% Nonidet P40 (v/v), the batches are passed through a membrane using centrifugation for one minute at 10,000 x g in a plastic column containing a polypropylene frit for mechanical support on which a membrane for binding the nucleic acids is fixed by means of a lock ring.

1. Pall Fluoro Trans G, Poly(vinylidene difluoride), hydrophobic, pore size 0.2 µm,
2. GORE-TEX polyester fleece 9318, polytetrafluoroethylene, hydrophilic, pore size 3 µm,
3. Millipore Fluoropore PTFE, polytetrafluoroethylene, hydrophobic, pore size 3 µm,

are used as membranes.

The material having passed through is collected in a collecting tube and discarded. The membranes are washed successively with 600 µl of a buffer containing guanidinium thiocyanate and with a buffer free of guanidinium thiocyanate where each of the wash buffers is passed through the membrane by centrifuging at 10,000 x g. Subsequent to the second washing, the membranes are centrifuged to dryness for 2 min at 20,000 x g. Thereafter, the RNA is eluted from the membrane by pipetting 70 µl of water onto the membrane and incubating for 2 min at room temperature. The eluate is pipetted from the top of the membrane using a pipette. The elution is repeated with another 70 µl of water, and the eluates are combined.

The amount of isolated RNA is determined by measuring the light absorption at a wavelength of 260 nm, and the purity of the RNA is established by determining the ratio of light absorption at 260 nm and 280 nm (see Table 9).

ammonioethyl)ammonium bromide in 200 mM sodium citrate pH 3.0

and stored for 48 hours at room temperature. All of the batches were conducted as double determinations.

To isolate the RNA, the samples are centrifuged for 2 min at 1,000 x g, the supernatant is decanted, and the pellet is dissolved in 700 µl of a solution of 6 M guanidine hydrochloride, 200 mM Tris-HCl, pH 7.0, and 1% (v/v) Nonidet P40. Thereafter, 80 µg of proteinase K is added, and the batches are incubated for 30 min at 40°C. 350 µl of acidic phenol is added each time, and the batches are extracted by vigorous agitation. Following addition of 350 µl of chloroform and another extraction, the batches are centrifuged for 3 min at 14,000 x g to effect phase separation. The aqueous phase is removed and extracted once more with 700 µl of chloroform. Following another centrifugation, the aqueous phase is removed again, and the RNA is precipitated by adding 70 µl of 3 M sodium acetate, pH 5.2, and 700 µl of isopropanol over 30 min at -20°C. The RNA is centrifuged off over 10 min at 20,000 x g, the supernatant is removed, the pellet is washed once with 600 ml of 80% (v/v) ethanol, subsequently dried and redissolved in 100 µl of water free of RNase.

The amount of isolated RNA is determined by measuring the light absorption at a wavelength of 260 nm, and the purity of the RNA is established by determining the ratio of light absorption at 260 nm and 280 nm (see Table 10).

Table 10: RNA yield and purity. To determine the yield, the calculation factor for RNA is used (1 OD_{260nm} = 40 µg/ml). Double determinations are carried out each time.

yield, the calculation factor for RNA is used (1 OD_{260nm} = 40 µg/ml).

Substance	Concentration in % (w/v)	Yield (µg)
Ethanediyl-1,2-bis(dimethyldecylammonium bromide)	1 9 15	22.2 25.0 19.5
Ethanediyl-1,2-bis(dimethyldecylammonium thiosulfate)	1 9 15	24.5 25 24
Ethanediyl-1,2-bis(dimethyldecylammonium sulfate)	1 9 15	22.2 25 22.4
Ethanediyl-1,2-bis(dimethyldecylammonium iodide)	1 9 15	18.9 23 19.2
N,N',N"-tridecyl-N,N,N',N",N"-pentamethyl-bis-(2-ammonio-ethyl)ammonium bromide	1 9 13	15.3 12.7 23.2
N,N',N"-tritetradecyl-N,N,N',N",N"-pentamethyl-bis-(2-ammonioethyl)ammonium bromide	1 9 15	11.3 9.9 6.3
Ethanediyl-1,2-bis(dimethyloctylammonium bromide)	3 15	7.6 6.5
Propanediyl-1,2-bis(dimethyldecylammonium bromide)	3 8 15	21.2 24.6 24.7
Butanediyl-1,2-bis(dimethyldecylammonium bromide)	1 9 13	24.6 25 11.3
Ethanediyl-1,2-bis(dimethyldodecylammonium bromide)	1 8 15	14.4 14.5 7.3
Propanediyl-1,2-bis(dimethyltetradecylammonium bromide)	1 9 15	13.8 18 14.6
Hexadimethrine bromide	1 5	9.6 3

The results show that all of these substances can be used in complexing RNA. Under the selected conditions, however, some of these substances work significantly more

to separate the reporter dye from the quencher dye, thereby generating a sequence-specific fluorescence signal which increases with every amplification cycle. The quantification is based on the threshold cycle wherein a previously defined fluorescence limit is reached. A comparison of the threshold cycles provides a measure for the relative concentration of template in different samples. Measurement during the logarithmic phase, where PCR precision is a maximum, provides precise data for an accurate determination.

The results are illustrated in Table 12.

Table 12: Analysis of β-actin mRNA using the TaqMan™ RT PCR. The threshold cycles (C_T) of the TaqMan™ evaluation are illustrated as a function of storage of the stabilized sample. Each sample was subjected to a double determination in the ABI PRISM 7700 Sequence Detector.

Storage	C_T
24 hours 4°C	17.23 18.51
96 hours 4°C	18.30 18.29
24 hours room temperature	17.93 17.89
96 hours room temperature	19.34 19.35

containing guanidinium thiocyanate and ethanol. Then, 80 µl of Tris-HCl buffer containing $MgCl_2$ and 75 U of DNase I (Pharmacia) is pipetted on the silica membrane and incubated for 15 min at room temperature to degrade the genomic DNA. The spin column is washed once more with 350 µl of said buffer containing guanidinium thiocyanate and ethanol, and subsequently with 500 µl at a time of a wash buffer containing ethanol. The spin column is centrifuged to dryness for 3 min at 20,000 x g, and the RNA is eluted in two steps using 50 µl of water each time. 4 µl of this eluate at a time is employed in an RT PCR detection of β-actin mRNA in an ABI PRISM 7700 Sequence Detector (Applied Biosystems). The reaction conditions for the RT PCR detection are identical to those described in Example 12. 30 µl of the eluate at a time is separated in a 1.2% agarose/formaldehyde/MOPS gel. The results are illustrated in Table 13 and Figure 1.

Table 13: Analysis of β-actin mRNA using the TaqMan™ RT PCR. The threshold cycles (C_T) of the TaqMan™ evaluation are illustrated as a function of storage time of the stabilized samples and the controls. Each sample was subjected to a double determination in the ABI PRISM 7700 Sequence Detector.

Storage	Stabilized sample	Control
10 min RT	15.90 16.17	21.49 22.16
24 hours 4°C	16.25 15.82	40" 40"
48 hours 4°C	16.43 16.49	40" 40"

In the control, the RNA is incubated for about 10 seconds in the plasma in an unprotected state before the stabilization buffer is added and incubation is

1, lanes 4 and 5), nor in a β-actin TaqMan RT PCR (Controls) where a threshold of 40 indicates that no amplification signal has been generated during 40 PCR cycles and thus, no β-actin mRNA has been detectable.

In contrast, both the results of β-actin mRNA amplification and the results of gel analysis (Fig. 1) indicate that no degradation of RNA has occurred in the stored samples added with stabilization buffer (stabilized samples 10 min RT, 24 hours 4°C, 48 hours 4°C). This can be seen in the clearly visible bands of ribosomal RNA in the gel and in the TaqMan RT PCR C_T values which, considering the accuracy limits of this method, must be referred to as constant.

This result is also confirmed in Example 14 wherein a highly sensitive detection of β-actin mRNA in the blood sample by means of TaqMan™ RT PCR is possible even after storage for 96 hours at room temperature.

It has been demonstrated both for plasma and blood that it is possible to protect RNA in these biological samples from degradation by using cationic compounds. In contrast, unprotected RNA is completely degraded within a few seconds in both sample materials.

Example 16: Isolation of HeLa-RNA from plasma, using Ethanediyl-1,2-bis(dimethyldecylammonium bromide), buffered with citric acid in a pH range from 3-7

15 µg of HeLa-RNA was spiked into 500 µl plasma and mixed with 500 µl of a buffer, containing 2 % (w/v) Ethanediyl-1,2-bis(dimethyldecylammonium bromide) and 0.5 M citric acid of different pH-values (range from pH 3 to 7) and thereafter incubated for 10 minutes at room temperature. For the RNA isolation, the complexes consisting of the cationic substance and the nucleic acids, were pelleted by centrifugation at 1100 x g for 3 minutes and the pellet was

buffered with citric acid in a pH range from 3-5

15 µg of HeLa-RNA was spiked into 500 µl plasma mixed with 500 µl of a buffer, containing 2 % (w/v) Ethanediyl-1,2-bis(dimethyldecylammonium bromide) and 0.5 M citric acid of different pH-values (range from pH 3 to 5) and incubated for 10 minutes at room temperature for 24 and 48 h at 4°C, respectively. For the isolation of the RNA, the complexes consisting of the cationic substance and the nucleic acids were pelleted by centrifugation at 1100 x g for 3 minutes and the pellet was subsequently resolved in 600 µl of a buffer containing 6 M guanidine hydrochloride, 1 % (v/v) Nonidet-P40 and 50 mM Tris HCl pH 7,0. 800 µg Proteinase K were added and the sample was incubated at 40°C for 30 minutes. Then 600 µl of a solution, containing 80 % (v/v) ethanol and 10 % (v/v) Nonidet-P40 was added and the sample was applied to spin column containing silica membrane via centrifugation. The membrane was washed once with a buffer containing guanidine thiocyanate and ethanol and once with a buffer containing sodium cloride and ethanol. The silica membrane was dried by centrifugation at 20 000 x g for 3 minutes. RNA was eluted form the silica membrane with 100 µl of RNAse free water by centrifugation. 30 µl of the respective eluate were applied on a 1.2 % (w/v) agarose/formaldehyde gel.

As a negative control experiment (K), the HeLa-RNA was spiked directly into 500 µl of plasma and after ten seconds, 500 µl of a buffer, containing 2 % (w/v) Ethanediyl-1,2-bis(dimethyldecylammonium bromide) and 0.5 M citric acid pH 3.0 was added. The sample was incubated additional 10 minutes and the RNA was isolated as described above.

In Figure 3 an agarose/formaldehyde gel picture shows the isolated RNA bands at different pH-values, whereas the samples were incubated 10 minutes at room temperature,

gel pictures show the isolated RNA bands for the five cationic substances A, B, C, D and E used:

A: o-Xylylene-bis-decyldimethylammonium bromide
B: m-Xylylene-bis-decyldimethylammonium bromide
C: p-Xylylene-bis-decyldimethylammonium bromide
D: [1,8]-dimethylnaphthaleno,alpha,alpha'-bis-dimethyldecylammonium bromide
E: Ethanediyl-1,2-bis(decyldimethylphosphonium bromide)

The experiment shows, that the cationic substances can be used to isolate RNA from plasma. The yields of the spiked RNA were between 63 % (=3,2 µg) and 74 % (=3.7 µg).

Example 19: Isolation of RNA and genomic DNA from 1 x 10^6 HeLa cells, using cationic substances with two nitrogen- or phosphor centers, linked by a bridge consisting of an aromatic compound or ethane

1 x 10^6 HeLa cells were dissolved in 500 µl PBS buffer and mixed with 500 µl of a solution of the cationic substance A, B, C, D or E (see below) and incubated for 10 minutes at room temperature. For the isolation of the RNA, the complexes consisting of the cationic substance and the nucleic acids were pelleted by centrifugation at 1530 x g for 3 minutes and the pellet was subsequently resolved in 300 µl of a buffer containing 6 M guanidine hydrochloride, 1 % (v/v) Nonidet-P40 and 50 mM Tris HCl pH 7,0. 400 µg Proteinase K were added and the sample was incubated at 40°C for 10 minutes. Then 300 µl of a solution, containing 80 % (v/v) ethanol and 10 % (v/v) Nonidet-P40 was added and the sample was applied to a spin column containing silica membrane via centrifugation. The spin column was washed once with a buffer containing guanidine thiocyanate and ethanol and once with a buffer containing sodium cloride and ethanol. The silica membrane was dried by centrifugation at

The DNA was eluted form the silica membrane with 100 µl of water by centrifugation. 25 µl of the eluate were analysed on a 0.8 % (w/v) agarose/TBE gel.

In Figure 6 a plurality of five agarose/formaldehyde gel pictures show the genomic DNA bands for the five cationic substances A, B, C, D and E used: *

A: o-Xylylene-bis-decyldimethylammonium bromide
B: m-Xylylene-bis-decyldimethylammonium bromide
C: p-Xylylene-bis-decyldimethylammonium bromide
D: [1,8]-dimethylnaphthaleno,alpha,alpha'-bis-dimethyldecylammonium bromide
E: Ethanediyl-1,2-bis(decyldimethylphosphonium bromide)

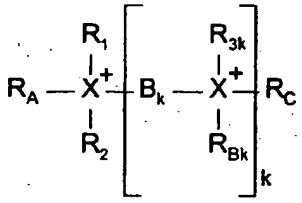
The yields of genomic DNA from 0.5 ml of blood are in the range of 6 µg to 11 µg

Example 21: Stabilization of RNA in plasma, using cationic substances with two nitrogen- or phosphor centers, linked by a bridge consisting of an aromatic compound or ethane, buffered with tartaric acid

6 µg of HeLa-RNA was spiked into 500 µl plasma mixed with 500 µl of a buffer, containing the cationic substance A, B, D or E (see below) in a concentration of 4 to 5 % (w/v) and 0.25 M tartaric pH 4 and stored for 24 h at room temperature. For the RNA isolation, the complexes consisting of the cationic substance and the nucleic acids were initially pelleted by centrifugation at 1530 x g for 3 minutes and the pellet was subsequently resolved in 300 µl of a buffer containing 6 M guanidine hydrochloride, 1 % (v/v) Nonidet-P40 and 50 mM Tris HCl pH 7.0. 400 µg Proteinase K were added and the sample was incubated at 40°C for 10 minutes. Then 300 µl of a solution, containing 80 % (v/v) ethanol and 10 % (v/v) Nonidet-P40, was added and the sample was applied to a spin column containing silica

Claims:

1. A method of stabilizing and/or isolating nucleic acids from a biological sample, comprising the following step:
contacting the biological sample with at least one cationic compound of formula (I)



(I)

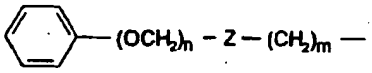
wherein conjugated bases of strong and/or weak inorganic and/or organic acids are used as anion (A), and wherein the substance consisting of cationic compound (I) and anion is neutral in charge on the whole,

and wherein

X represents nitrogen (N) or phosphor (P),

k represents the integer 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24,

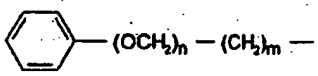
B_k represents aliphatic alkanediyl bridges which may be substituted on none, on one or more carbon atoms, and wherein one or more non-adjacent carbon atoms may be replaced by oxygen, and which have the structure



wherein n, m independently represent the integers 0, 1, 2, 3, 4, 5, or 6, and

Z represents one of the structures -O-, -CO-, -CO_2-, -OCO-, -CO-N-, -N-CO-, -O-CO-N-, -N-CO-O-, -S-, or -S-S-;

or R_1, R_2, R_{3k} represent phenyl, benzyl, phenoxyethyl having the structure



wherein n, m independently represent the integers 0, 1, 2, 3, 4, 5, or 6;

R_A, R_{Bk}, R_C, which may be identical or different and which may be unsubstituted or substituted on one or more carbon atoms, represent hydrogen, linear or branched C_1-C_{21} alkyl, linear or branched C_1-C_{21} alkenyl, linear or branched C_1-C_{21} alkynyl, or a structure

$$CH_3-(CH_2)_n-Z-(CH_2)_m-$$

wherein n, m independently represent the integers 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, and

Z represents one of the structures -O-, -CO-, -CO_2-, -OCO-, -CO-N-, -N-CO-, -O-CO-N-, -N-CO-O-, -S-, or -S-S-;

alternatively, R_A and R_C together form a residue R_{AC} and

R_1, R_2 and R_{3k}, which may be identical or different, represent

the C_1-C_6 alkyl groups methyl, ethyl, propyl, isopropyl, butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, and/or 1-ethyl-2-methyl-propyl,

and/or the C_3-C_6 alkenyl groups 2-propenyl (allyl), 2-butenyl, 3-butenyl, 1-methyl-2-propenyl, 2-methyl-2-propenyl, 2-pentyl, 3-pentyl, 4-pentyl, 1-methyl-2-butenyl, 2-methyl-3-butenyl, 3-methyl-3-butenyl, 1,1-dimethyl-2-propenyl, 1,2-dimethyl-2-propenyl, 1-ethyl-2-propenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-methyl-2-pentenyl, 4-methyl-2-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 3-methyl-3-pentenyl, 4-methyl-3-pentenyl, 1-methyl-4-pentenyl, 3-methyl-4-pentenyl, 4-methyl-4-pentenyl, 1,1-dimethyl-2-butenyl, 1,1-dimethyl-2-butenyl, 1,1-dimethyl-3-butenyl, 1,2-dimethyl-2-butenyl, 1,2-dimethyl-3-butenyl, 1,3-dimethyl-2-butenyl, 1,3-dimethyl-3-butenyl, 2,2-dimethyl-3-butenyl, 2,3-dimethyl-2-ethyl-2-butenyl, 2-ethyl-3-butenyl, 1,1,2-trimethyl-2-propenyl, 1-ethyl-1-methyl-2-propenyl, and/or 1-ethyl-2-methyl-2-propenyl,

and/or the C_3-C_6 alkynyl groups 2-propynyl (propargyl), 2-butynyl, 3-butynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 3-methyl-2-butynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, 5-hexynyl, 3-methyl-2-pentynyl, 4-methyl-2-

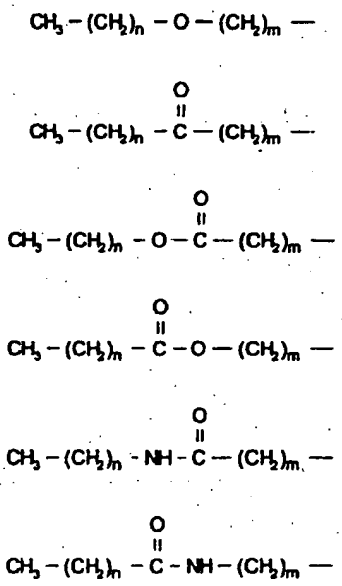
and/or a structure

$$CH_3-(CH_2)_n-Z-(CH_2)_m-$$

wherein n, m are independent of each other, and n represents the integer 2, 3 or 4, m represents the integer 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18, and

Z represents one of the structures -O-, -CO-, -OCO-, -CO-N-, or -N-CO-.

3. The method according to claim 1 or 2, wherein one or more of the groups designated as R_A, R_{Bk} and R_C represent one of the structures

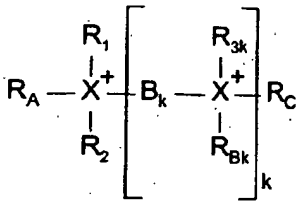


or

4. The method according to any of the preceding claims, wherein R_1, R_2 and/or R_{3k} represent an allyl

hydroxyl groups, and/or one or more -SH, $-NH_2$, -NH-, and/or =N- groups, and wherein the substituents may be identical or non-identical to each other.

8. The method according to claim 7, wherein one or more carbon atoms of the groups R1, R2, R3k, RA, RBk, and RC, which are not directly bound to one of the nitrogen atoms or phosphor atoms in compound



(I)

are substituted.

9. The method according to any of the preceding claims, wherein the aliphatic and/or aromatic carbon atoms of the bridging groups B_k are substituted with one or more halogen atoms, particularly fluorine atoms, and/or one or more primary, secondary and/or tertiary hydroxyl groups, and/or one or more -SH, $-NH_2$, -NH- and/or =N- groups, and/or one or more linear or branched C_1-C_4 alkyl groups, and wherein the substituents may be identical or non-identical to each other.

10. The method according to claim 9, wherein the aliphatic and/or aromatic carbon atoms of the bridging groups B_k are substituted with one or more methyl, ethyl, propyl, i-propyl, butyl, 2-methylpropyl, and/or tert-butyl groups.

11. The method according to one or more of the preceding claims, wherein ethanediyl-1,2-bis(dimethyldecyl-

19. The method according to any of the preceding claims, wherein said mixing is followed by incubating, preferably for 10 minutes at room temperature, in order to isolate nucleic acids directly.

20. The method according to any of the preceding claims, wherein said at least one cationic compound and/or the complex formed of nucleic acid and cationic compound(s) is added with additional means to support lysis and/or means for homogenization and/or means for mechanical exposure and/or means for enzymatic exposure.

21. The method according to claim 20, wherein the means used to support lysis are alcohols, particularly branched or unbranched C1- to C4 alkanols, such as isopropanol, aldehydes, particularly lower C1- to C4-aldehydes, branched or unbranched, such as glyoxal, phenols, phenol derivatives, ionic, zwitterionic and non-ionic detergents, reagents reducing sulfhydryl, particularly dithiothreitol, phosphoric acid derivatives, particularly tributyl phosphate, chaotropic reagents such as urea, carboxylic acids, such as citric acid or malonic acid, or plain salts, such as ammonium salts or alkali phophates, which may be present alone or in combination.

22. The method according to any of the preceding claims, wherein the complexes formed of nucleic acid and cationic compound(s) are sedimented by centrifuging.

23. The method according to claim 22, wherein centrifugation is conducted at low g values, particularly from 500 to 5000 g for 3-10 minutes.

24. The method according to claim 22 and/or 23, wherein the complex in the sediment having formed is dissolved in a buffer.

- collecting the complexes of nucleic acid and cationic compound on the bottom of a vessel or on a membrane using centrifugation, vacuum, excess pressure and/or capillary forces,

- optionally washing the complexes with a suitable wash solution using centrifugation, excess pressure, vacuum and/or capillary forces,

- optional addition of a suitable reagent solution,

- dissolving the complexes to liberate the nucleic acids under non-binding or binding conditions, and

- isolating the liberated nucleic acid.

29. A kit for stabilizing and/or isolating nucleic acids, comprising at least one cationic compound as defined in one or more of the preceding claims 1-14.

30. The kit according to claim 29, additionally comprising suitable buffers.

31. The kit according to any of claims 29 and/or 30, said kit additionally including suitable means to support lysis and/or means for purification and/or means for enzymatic exposure and/or means for mechanical exposure.

32. Use of a compound as defined in one of claims 1-14 for stabilizing and/or isolating nucleic acids.

33. Use of a compound as defined in one of claims 1-14 in an automatized process for stabilizing and/or isolating nucleic acids.

34. A complex, comprising of a nucleic acid and at

Figure 1: RNA Integrity

All samples are prepared in double determinations. 30 µl of eluate at a time is separated on a 1.2% agarose/formaldehyde/MOPS gel.

Lane 1: Length marker 0.24 - 9.5 kb
Lane 2/3: HeLa RNA stabilized with ethanediyl-1,2-bis(dimethyldecylammonium bromide)
Lane 4/5: Control

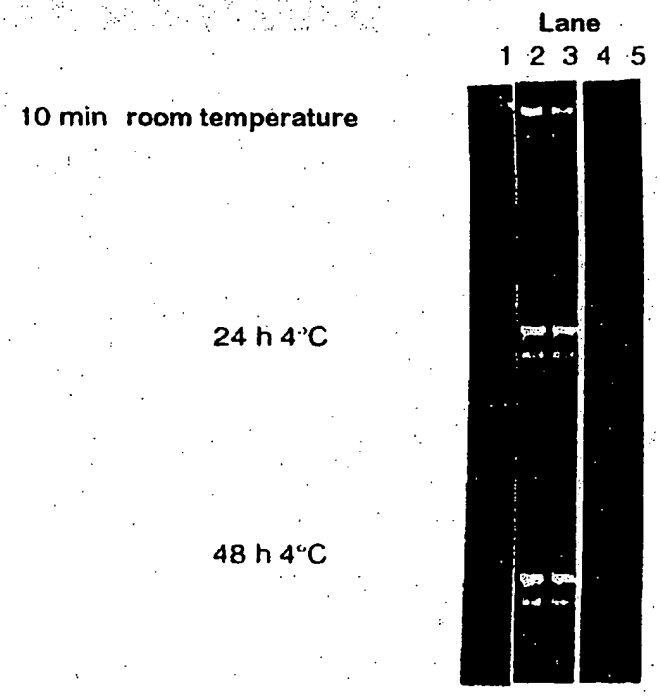


Figure 3:

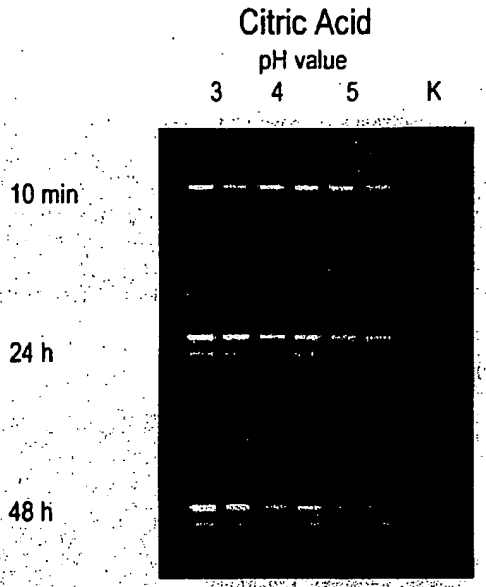


Figure 4:

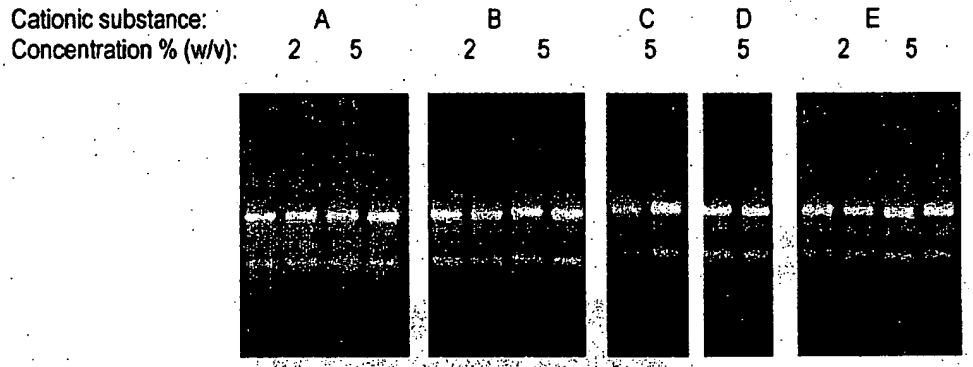
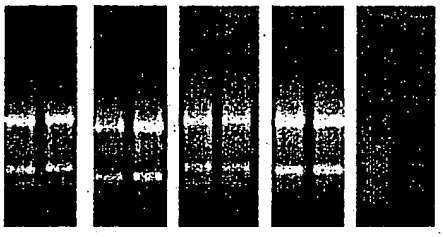


Figure 7:

Cationic substance:	A	B	D	E	K
Concentration % (w/v):	2	2	5	2	



889.
ENZYME KINETICS BY POLARIMETRY: A STUDY OF THE PH DEPENDENCE AND METAL ION INHIBITION OF INVERTASE-CATALYZED SUCROSE HYDROLYSIS. *Tracey L Harshberger, and John A Cramer, Department of Chemistry, Seton Hill University, 1 Seton Hill Drive, Greensburg, PA 15601, Fax: 724-830-1571*

A convenient kinetic assay has been developed for monitoring the invertase-mediated hydrolysis of sucrose using an automatic digital polarimeter operating in the time mode. Invertase, derived from yeast, catalyzes the hydrolysis of sucrose to form an equal molar mixture of D-glucose and D-fructose monosaccharides. Since sucrose is dextrorotatory and the monosaccharide product mixture is levorotatory an inversion of optical activity is observed as the reaction proceeds. An examination of the catalytic activity of invertase as a function of pH was performed which demonstrated that invertase has a broad pH range of optimal activity centered around pH 4.5. Data from a study of the inhibitory effects of various metal ions on invertase activity will be presented as well.

890.
EPR STUDY OF THE DNA BINDING OF A CISPLATIN ANALOGUE. *Laura F. Millemon, Barry W. Hicks, and Kimberly A. Gardner, Department of Chemistry, U. S. Air Force Academy, 2355 Fairchild Suite 2N225, USAF Academy, CO 80840-6230, C04Laura.Millemon@usafa.af.mil*

Cisplatin is a widely used chemotherapy for many types of cancer. Cisplatin binds to purine dimers in DNA and RNA and interferes with DNA replication and repair, and RNA transcription and translation ultimately culminating in cell death. We synthesized the spin-labeled cisplatin analogue, cis-di(4-aminotempo)dichloroplatinum(II) (PDN-1). PDN-1 was purified and characterized by IR spectroscopy, UV-Vis spectroscopy, C, H, N anal., LCMS, and ESR (EPR) spectroscopy. In previously published work with PDN-1 on whole cells, the EPR spectrum showed two components, one due to PDN-1 displaying isotropic diffusion (free PDN-1), and one due to PDN-1 with anisotropic motion (bound PDN-1). That work was published long before the mechanism of cisplatin was understood. We are using PDN-1 in in vitro studies with synthetic DNA and RNA and with liposomes to investigate the source of the anisotropic signal in the EPR spectrum, and to investigate the binding kinetics and thermodn. of PDN-1 to nucleotide polymers.

891.
EVALUATION OF DNA STABILITY IN AN IONIC LIQUID. *Aisha C. Robinson, James H. Davis Jr., and Naomi F. Campbell, Department of Chemistry, University of South Alabama, 307 University Blvd., CHEM 223, Mobile, AL 36608, Fax: 251-460-7359*

Ionic liquids are molten salts, liquids that are composed entirely of ions. They have virtually no vapor pressure and high ionic conductivity. Ionic liquids have been used as replacements for volatile organic solvents for numerous materials because of their very low vapor pressure and ease of recycling. They are being championed as the new "green" solvent because of their benign affect on the environment. The physiological action of many drugs involves the intercalation of the drugs into the duplex DNA. This project explores the effect of the stability of the DNA duplex in the presence of an ionic liquid. Calf thymus DNA was solubilized in an imidazole based ionic liquid and the stability of the duplex was examined.

892.
EVIDENCE OF A HOMOSERINE ESTER AS A BY-PRODUCT OF PROTEIN CLEAVAGE BY CYANOGEN BROMIDE. *Matifadza G. Hlatshwayo[1], Thomas H. Morton[2], and Jacqueline Bennett[1]. (1) Department of Chemistry, Drury University, 900 North Benton Avenue, Springfield, MO 65802, mhlatshw@drury.edu, (2) University of California, Riverside*

A nonenzymatic reaction that cleaves a specific peptide bond uses the reagent cyanogen bromide which specifically cleaves the peptide bond at the carbonyl side of methionine residues. It has been in use in this capacity for more than 40 years. In addition to the generally accepted products of this reaction, recent work suggests that another side reaction may be occurring. This proposed reaction involves the displacement of methyl thiocyanate by the amide carbonyl oxygen from the adjacent N-terminal amino acid. The resulting product would yield an uncleaved protein containing a homoserine ester. In order to prove that this proposed mechanism occurs, a synthetic peptide containing norrnethionine, rather than methionine is currently being studied. The expected product from cyanogen bromide treatment of this peptide should occur via the newly proposed mechanism due to ring constraints. The crude mix has been purified using HPLC and peak-containing fractions submitted for mass spectrometric analysis at the University of California, Riverside. After the existence of the homoserine ester has been proven using the norrnethionine contalning synthetic peptide, it is our goal to establish its existence within regular methionine containing peptides and to eventually develop reaction conditions to limit its occurrence.

893.
EXAMINATION OF PHOTOACTIVATION IN DNA PHOTOLYASE USING DIFFERENCE INFRARED SPECTROSCOPY. *M. Cabell Jonas, Lori A. McKee, and Sunyoung Kim, Biochemistry, Virginia Polytechnic Institute and State University, 124 Engel Hall, Blacksburg, VA 24061, Fax: 540-231-9070, mjonas@vt.edu*

DNA mutations caused by increasing ultraviolet radiation can have lethal effects on all biological organisms. DNA photolyase is a flavoenzyme that can utilize the energy from visible light to repair UV-damaged DNA, specifically pyrimidine dimers. Two processes are important for dimer repair: photoactivation and photorepair. During photoactivation, the catalytic cofactor FAD· is reduced to FADH· by the transfer of an electron through a chain of amino acids in photolyase. Once reduced, FADH· can then transfer an electron to the pyrimidine dimer to carry out photorepair. We propose to map the molecular interactions of photoactivation using difference FTIR spectroscopy. In our difference infrared spectrum, we observe the vibrational modes from both the catalytic cofactor and aromatic amino acids in photolyase. These data allow us to examine the dynamic structural changes that occur within the protein matrix that correspond to the redox changes of FAD.

894.
EXPRESSION OF CYCLIN B1 IN P53-MUTANT U138MG GLIOBLASTOMA CELLS AFTER EXPOSURE TO ETOPOSIDE (VP16). *David Stroup, and Lori A. Wetmore, Department of Chemistry, William Jewell College, 500 College Hill, Liberty, MO 64068, stroupd@william.jewell.edu*

Unsuccessful glioblastoma brain cancer treatment is linked to dysfunctional G_2/M cell cycle transition. P53 protein detects DNA damage, and regulates cell cycle progression, and Cyclin B1 expression controls entrance into mitosis at G_2/M. U138MG, a p53-mutant glioblastoma cell line, displays abnormal G_2/M cell cycle regulation. In U138MG with DNA damage, the role of cyclin B1 in cell cycle control at G_2/M can be elucidated, using etoposide, an anti-cancer agent that causes DNA double strand breakage; caffeine, an ATM pathway inhibitor, and genistein, an ATM pathway activator. Thus a testable hypothesis is U138MG cells dosed with etoposide will display increased Cyclin B1 expression in the presence of caffeine and decreased expression in the presence of genistein. Cyclin B1 expression was detected using immunohistochemistry with hematoxylin counterstaining. Low overall Cyclin B1expression was detected microscopically in U138MG cells; however, etoposide and caffeine generated consistantly higher levels of Cyclin B1 expression compared to control.

895.
FLANKING SEQUENCES MODULATE DIEPOXIDE CROSS-LINKING EFFICIENCIES AT THE 5'-GNC SITE. *Gregory A. Sawyer, Chemistry, Colby College, 5700 Mayflower Hill Drive, Waterville, ME 04901, Fax: 207-872-3804, gasawyer@colby.edu, and Julie T. Millard, Department of Chemistry, Colby College*

Diepoxybutane, diepoxyoctane, and mechlorethamine are cytotoxic DNA cross-linking agents that vary in their carcinogenic versus chemotherapeutic potentials. Interstrand cross-linking occurs between the N7 positions of deoxyguanosine residues on opposite strands of the DNA duplex. Each synthetic DNA oligomer used in this study contains four 5'-N1GN2CN3 sites (within a 32 base sequence), and we are currently systematically varying the bases in the N1 and N2 positions to determine the resulting cross-linking efficiencies of each cytotoxic agent. Each duplex is 5'-end labeled and incubated with cross-linking agent. Interstrand cross-links are purified through denaturing polyacrylamide gel electrophoresis and then subjected to piperidine cleavage. The amount of cleavage at each deoxyguanosine residue is determined by sequencing gel